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Amylopectin chain length distribution in grains of japonica rice as affected by nitrogen fertilizer and genotype

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ABSTRACT

This study investigated the chain length distribution (CLD) of two japonica rice cultivars under six nitrogen (N) treatments by high performance size exclusion chromatography, with the aims to elucidate the effect of N on rice quality and its biological mechanism. Results showed significant influence of N on CLD. In comparison with low N rate, high N lowered the percentage of short amylopectin branches. Fitting with the CLD model of Wu-Gilbert, it suggested that relative activity of SBE to SS was lower at high N rate, thus producing fewer short amylopectin branches. Comparison of CLD between N rates and between cultivars revealed that decrease in short amylopectin branches or the relative ratio of short to long amylopcetin branches correlated with increase in flour gelatinization temperatures (T_o, T_p, and T_c) and decrease in pasting values (except PaT) and amylose-lipid gelatinization temperatures. In addition, quality traits of Wuyujing3, a cultivar with premium eating quality, expressed stably across N treatments compared with the high-yielding cultivar Wuyunjing7.

1. Introduction

Rice (*Oryza Sativa.* L) is growing in importance outside Asia as considerable growth in its consumption in Africa and South America (Muthayya et al., 2014). Generally, rice varieties in Asia are classified into two major types, indica and japonica. Japonica rice is preferred by people in northern Asia due to its sensory quality of soft texture and moderate stickiness (Sun et al., 2011). China is the largest producer and consumer of japonica rice. Along with rise in living standard, rice quality, eating and cooking quality in particular has become prime consideration of both rice producers and researchers like geneticists, breeders, and agronomists. However, our current knowledge of the physicochemical factors affecting quality of japonica rice is still incomplete, as is reflected by the persistence of benchmark varieties of Koshihikari (Fitzgerald et al., 2009) and Wuyujing3 (Yang et al., 2013) for many decades in spite of the grain yield achieved over decades.

Physicochemical foundation of rice eating and cooking quality has been investigated extensively. Results showed that chemical components including amylose, amylopectin, proteins, and lipids coordinately control physical properties of cooked rice and subsequently the eating quality (Bhattacharya, 2011). There has been an increasing awareness of the role of amylopectin fine structure in physicochemical properties of rice. Amylopectin contributes to swelling of starch granules and pasting, as measured by Rapid Visco Analyzer (RVA). Jane et al. (1999) reported that long chains of AP (DP > 37) were positively associated with starch pasting temperature (PaT) but negatively with peak viscosity (PKV) and breakdown (BDV). By contrast, Yang et al. (2013) found no significant relationship between amylopectin structure and RVA parameters. Amylopectin also plays a substantial role in the process of starch gelatinization and retrogradation. Using differential scanning calorimeter (DSC), numerous studies showed that short amylopectin A chain (DP 6-12) was negatively correlated to the gelatinization temperature (T_o, T_p, T_c) and enthalpy (ΔH_g) during flour gelatinization (Nakamura et al., 2002; Vandeputte et al., 2003), while the long amylopectin chains exhibited the opposite trends

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(Jane et al., 1999). In addition, Wang and Wang (2002) investigated retrograding behavior of waxy rice starch, and attributed the low retrogradation tendency in waxy rice starches to a larger amount of A chain and a shorter exterior chain length of amylopectin.

Nitrogen (N), a primary element demand for rice grain, has obvious effect on eating and cooking quality of rice. It is well known that nitrogen application increase protein content and decrease amylose content in rice grain (Dong et al., 2007). Gunaratne et al. (2011) showed that nitrogen fertilizer application increased pasting onset temperature (PaT), cold paste viscosity (CPV) and setback viscosity (SBV), but lowered peak viscosity (PKV), breakdown viscosity (BDV), peak temperature (T_p). In contrast, Singh et al. (2011) reported that all gelatinization parameters were obviously enhanced. With respect to rice sensory quality, Champagne et al. (2009) reported that N fertilizer significantly worsen the palatability of cooked rice, causing it hard, rough and stick in texture as the results of increase in protein content and decrease in amylose content, as was also reported by Singh et al. (2011). On the other hand, Gunaratne et al. (2011) found that N fertilizer reduced the gel hardness of rice flour but increased the gel cohesiveness. Therefore, N effect on physical properties and sensory qualities of rice is still unclear. In addition, little is known concerning the effect of N on rice quality from perspective of amylopectin fine structure.

In Southern China, one of the main areas for japonica rice planting, rice production is characterized as being high yielding and high N input. For example in Jiangsu Province, the average N rate in rice is 270 kg/ha and even exceeds 300 kg/ha in many counties (Xue et al., 2013). Excessive use of nitrogen fertilizer has been a serious problem in this area, posing not only a threat to environment but also negative impact on rice eating and cooking quality. However, its physicochemical foundation is largely unknown, especially in knowledge of N influence on amylopectin fine structure and it biosynthesis mechanism.

Recently, Wu et al. (2013) developed a model of amylopectin fine structure (chain-length distribution, CLD) in cereal endosperm, with the aim to link CLD to concerted action of three key enzymes (starch synthase, SS; starch branching enzyme, SBE; and starch debranching enzyme, DBE). In the current study, CLD of two japonica rice cultivars under six N treatments was analyzed by high performance size exclusion chromatography (HPSEC) with refractive index detection, with the aims of (1) uncovering the variations of rice amylopectin structure with N and genotype, (2) clarifying the contribution of CLD to physicochemical properties of rice flour, and (3) exploring the biological mechanism of N effect on rice quality by the Wu-Gilbert model. We hope that these findings will further our understanding of physicochemical foundation of rice quality and be helpful for breeding and cultivation.

2. Materials and methods

2.1. Plant material and experiment design

Two japonica rice cultivars, Wuyujing3 and Wuyunjing7, were selected here. Wuyujing3 is famous for premium eating quality, while Wuyunjing7 is high-yielding but with medium eating quality (Suppl. Table S1 and Fig. S1) (Wei and Tang, 2011). Field experiment of nitrogen treatments was conducted at the experimental station of Nanjing Agricultural University (31°54′31″N, 119°28′21″E) in 2011. The soil type was clay soil, containing 1.48 g/kg total N, 10.37 mg/kg available P, and 49.53 mg/kg exchangeable K. The experiment was laid out in a split plot design with three replications. Six N treatments of two N rates and three timings of topdressing were conducted as follows: (1) LN1, LN2 and LN3, low N rate (75 kg/ha) and topdressing at initiation of panicle differentiation (as spikelet-promoting fertilizer), two weeks after initiation of panicle differentiation (as spikelet-sustaining fertilizer) and booting stage (as grain-filling fertilizer), respectively; (2) HN1, HN2 and HN3, high N rate (150 kg/ha). In addition, we use N0 as control, for which no N fertilizer was applied during the whole growth stage, except that P and K fertilizer (calcium superphosphate 140 kg/ha and potassium chloride 186 kg/ha) were applied before transplanting. To avoid leaching, each plot (11.5 m^2 in area) was separated by ridges mulched by plastic film. Rice was sown in seedbeds on May 25, 2011, and transplanted on June 20, 2011. At maturity, about 100 panicles with similar maturity were harvest, and then naturally dried and stored in −4 °C.

2.2. Milled-rice appearance and chemical compositions

Grain weight (GW) was weighted using an analytical balance, and grain length and width ratio was determined using digital vernier calipers. The chalky rice ratio (CRR) was calculated as total grain number divided by chalky grain number. Head milled rice was ground into powder and stored in −20 °C for physicochemical analysis. Moisture content, total starch and crude lipids and apparent amylose content (AAC) were analyzed according to AACC procedures (AACC, 2000). Total protein content (TPC) was calculated as sum of the four fractions, albumin, globulin, prolamin, and glutelin, which were extracted and assayed according to the method reported by Ning et al. (2009).

2.3. Amylopectin chain length distribution

The preparation, debranching and analysis of amylopectin conducted by high-performance size-exclusion chromatography (HPSEC) were performed as detailed in our previous report (Yang et al., 2013). For quantitative analysis, the empirical division method were used here: firstly, the HPSEC data were transformed to the number distribution, and then the amylopectin chain length were subdivided into four categories: (1) A, $6 \leq \mathrm{DP} \leq 12$; (2) B_1, $13 \leq \mathrm{DP} \leq 24$; (3) B_2, $25 \leq \mathrm{DP} \leq 36$; (4) B_3, $37 \leq \mathrm{DP} \leq 60$; and which were described as short, medium, long, and very long, respectively. Consequently, the ratio of $(A + B_1)$ to $(B_2 + B_3)$ was used as index of short to long chains distribution of amylopcetin.

2.4. Modeling the amylopectin biosynthesis

As an alternative, the chain length distribution (CLD) of debranched amylopectin was fitted to Wu-Gilbert model, using a publicly available FORTRAN program package (Wu et al., 2013). The basic premise of the model is that the CLD of chains spanning two lamellae, short single-lamellar ($\mathrm{DP} \leq 30$) and long translamellar ($30 \leq \mathrm{DP} \leq 60$) branches, is controlled by two different enzyme sets. By fitting with this model, we get two categories of most informative categories of parameters. The first category of parameter is the ratio of activity of SBE to that of SS (β, β(i) & β(ii)). The second category of parameter is the relative contributions of each enzyme set. For example, h(ii/i), relative heights of the peak and shoulder in CLD number distribution, represents the relative contribution of enzyme set ii to that of set i. Before data fitting with Wu-Gilbert model, SEC weight distribution of debranched amylopectin $w_{de}(\log X)$ should be converted into number distribution $N_{de}(X)$ using the relation $w(\log X) = X^2 N(X)$ as described by Wang et al. (2014).

2.5. Starch particle size analysis

Milled rice of all the samples were grounded by mortar and pestle. Then proteins were isolated from rice flour by alkali